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PHYSIOLOGICAL SERIES

No. 12: THE ACTION OF YEAST FRACTIONS ON THE GROWTH OF RATS, BY CASIMIR FUNK and ARCHIBALD BRUCE MACALLUM

(REPRINTED FROM THE JOURNAL OF BIOLOGICAL CHEMISTRY, VOL XXVII)

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STUDIES ON GROWTH.

IV. THE ACTION OF YEAST FRACTIONS ON THE GROWTH OF RATS.

BY CASIMIR FUNK AND ARCHIBALD BRUCE MACALLUM.*

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(Received for publication, August 3, 1916.)

The close relationship existing between the beri-beri and growth problems suggests the possibility of a fractionation of the active substance along lines already used in the investigation of beri-beri. Accordingly phosphotungstic acid was selected for the first attempt to separate out a physiologically active fraction which would stimulate the growth of young rats. The experimental difficulties which have repeatedly been emphasized in the investigation of the beri-beri vitamine, due to instability of this substance, were also encountered in the study on growth. The physiological activity of the different fractions diminishes with each manipulation and both the problems of beri-beri and of growth will not be solved until more suitable methods for the isolation of vitamine are available.

The results obtained so far clearly indicate that the growth-promoting substance is analogous to and possibly identical with the beri-beri vitamine and can be almost entirely precipitated with phosphotungstic acid. Subsequent fractionation of the residue obtained from the decomposed precipitate with silver nitrate and also with silver nitrate and baryta has shown that the precipitate containing purine bases and the filtrate from the silver nitrate and baryta precipitation are entirely negative as to their growth-promoting action; whereas the substances pre-

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cipitated with silver nitrate and baryta possess traces of the activity of the initial phosphotungstic acid precipitate. The experimental evidence indicates that considerably larger quantities of vitamines are necessary for stimulating growth than for curing beri-beri, and the losses occurring during fractionation are more apparent in the former than in the latter case. However, it must be admitted that while it is uncertain whether these two substances are chemically different, the results obtained do not exclude such a possibility. Lloyd's reagent, as recommended by Seidell,[1] has also been used as a precipitant without much success, as the rats on the filtrate have also shown increments in growth.

In the first instance autolyzed yeast was slightly acidified with hydrochloric acid and completely precipitated with phosphotungstic acid, carefully avoiding an excess of this reagent. After allowing the mixture to stand for 24 hours, the precipitate was collected on a Buchner funnel and repeatedly washed with a cold solution of phosphotungstic acid containing hydrochloric acid. The precipitate was then decomposed by the method described by Van Slyke,[2] with a mixture of amyl alcohol and ether and hydrochloric acid, only a small quantity of the precipitate remaining unchanged. After filtration of this small fraction the aqueous extract was evaporated *in vacuo* and the residue made up to a known volume and mixed in the diet in quantities calculated from the amount of autolyzed yeast necessary to promote growth. However, the quantity of this fraction had to be doubled and even tripled in order to obtain satisfactory results. The phosphotungstic acid filtrate was worked out in a similar way. This process offers the advantage that the yeast fraction is completely freed from substances soluble in lipoid solvents. The purine fraction was obtained from the phosphotungstic acid precipitate fraction by precipitation with silver nitrate and subsequent decomposition with sulfuretted hydrogen. The filtrate from the purine bases was precipitated with baryta in the usual way and the precipitate decomposed, freed from traces of baryta, evaporated, and the residue mixed with

[1] Seidell, A., *U. S. Public Health Report*, No. 325, 1916.
[2] Van Slyke, D. D., *J. Biol. Chem.*, 1915, xxii, 281.

the diet. The filtrate from the fraction containing vitamines was reprecipitated with phosphotungstic acid and the precipitate obtained after thorough washing, decomposed with amyl alcohol and ether. The results with the purine fraction and also with the silver nitrate-baryta filtrate are not included in this paper as they were entirely negative. The effect of the silver nitrate-baryta fraction was not sufficiently marked to encourage further investigation. The diet contained lard as the fat constituent, and 1 per cent sodium bicarbonate was added to neutralize the hydrochloric acid present in this fraction. Orange juice to the extent of 1 cc. a day was added to the drinking water to prevent the onset of scorbutic symptoms.

A large number of rats were kept on the above diets, especially on the phosphotungstic precipitate and filtrate and the records of only a few were selected for publication, in order to save space. Rats 9 and 10 were kept on phosphotungstic acid filtrate throughout the experiment. Rats 11 and 12 were changed after 34 days to the diet containing the phosphotungstic precipitate fraction which was followed by an improvement warranting the view that the growth-promoting substance is contained in this precipitate (Fig. 2 b).

Diets (Gm.).

	1.	2.
Casein	22	22
Sugar	10	10
Starch	23	23
Lard	30	30
Agar	2	2
Salts	2	2
$NaHCO_3$	1	1
Phosphotungstate precipitate		10
" filtrate	10	

Rat 9 (male) and Rat 10 (female).
0–96 days Diet 1.
28–100 " 1 cc. orange juice.

Rats 11 and 12. Males.
0–34 days Diet 1.
35–100 " " 2.
76–100 triple vitamine addition.

Days.	Weight.		Average daily food intake.	Days.	Weight.		Average daily food intake.
	9.	10.			11.	12.	
	gm.	gm.	gm.		gm.	gm.	gm.
0	35.2	41.8		0	37.9	34.2	
4	42.8	47.3	9.5	4	46.2	41.8	7.6
8	43.0	47.3	7.8	8	45.8	44.2	5.5
12	44.6	49.3	7.0	12	42.8	45.3	7.6
16	46.8	55.4	8.7	16	45.3	45.8	7.8
20	46.2	56.6	7.5	20	46.9	45.7	7.7
24	46.6	54.7	7.8	24	46.2	46.5	6.6
28	46.5	52.8	5.5	28	44.3	45.0	5.4
32	42.9	49.9	7.4	32	40.4	39.4	4.7
36	37.8	41.6	7.8	36	39.8	37.2	5.6
40	36.8	41.6	7.7	40	50.5	48.2	9.1
44		41.8	6.6	44	52.2	50.0	5.9
48		43.9	5.3	48	54.8	57.0	8.0
52		44.0	5.3	52	58.0	60.7	8.7
56		43.8	3.8	56	63.5	67.3	11.0
60		41.6	3.5	60	66.3	71.0	12.4
64		44.6	3.5	64	66.1	70.3	8.8
68		41.0	3.2	68	70.6	75.7	11.1
72		43.5	3.5	72	75.0	82.6	9.9
76		42.9	3.4	76	76.0	84.8	11.9
80		45.0	3.0	80	77.6	91.1	11.1
84		42.2	3.1	84	75.0	85.2	9.6
88		44.1	2.7	88	79.4	90.0	8.8
92		43.0	3.2	92	78.5	90.9	4.4
96		41.0	2.9	96	74.7	91.7	6.4
100				100	70.0	76.3	6.4

A second series of experiments was carried out on pressed yeast which had been heated with 10 per cent sulfuric acid at 90–95° for 6 hours. The hydrolysate was filtered, diluted with an equal volume of water, and precipitated with phosphotungstic acid. After standing for 24 hours the precipitate was filtered at the pump and well washed with 5 per cent sulfuric acid.

The precipitate was decomposed in the ordinary way with baryta. The final solution, slightly acid, was neutralized with sodium carbonate, carefully avoiding an excess, distilled *in vacuo*, standardized, and definite quantities were added to the diet. The

filtrate of the phosphotungstic acid precipitation was treated in a similar way.

The diet containing the substances precipitated by phosphotungstic acid was fed to four rats (Rats 80 to 83, Fig. 1) and enabled them to double their original weight after 32 to 36 days. This is about double the time required when yeast is the source of vitamines and the depreciation is due to the fractionation with the precipitating reagent.

Two rats (84 and 85, Fig. 2 a) were fed the diet containing the residue from the phosphotungstic filtrate. After 28 days they had added only a third to their original weight and had all the external symptoms of an acute deficiency. Then the diet with the precipitate was substituted and in 11 days they rapidly doubled their original weight and presented a normal appearance.

Diets (Gm.).

	1.	2.
Casein	22	22
Sugar	10	10
Starch	24	24
Fat (lard)	30	30
Salt	6	6
Agar	2	2
Residue phosphotungstic acid precipitate equal to	6	Dried yeast, 6
Residue phosphotungstic acid filtrate equal to		

Rats 80, 81, and 82. Males.
Rat 83. Female.
0–36 days Diet 1.

Days.	Weight.		Average daily food intake.	Weight.		Average daily food intake.
	80.	81.		82.	83.	
	gm.	gm.	calories	gm.	gm.	calories
0	24	25		20	30	
4	31	33	29 8	23 5	37	27 8
8	36	40 5	33 5	28	43	32 8
12	36	43	35 7	29	45	33 3
16	38	44 5	40 5	29	48	40 5
20	40	48	28 6	31	51	38 3
24	42	49	30 1	34	54	30 2
28	44 5	52	27 8	38	57	35 0
32	46	54	28 7	40	59	27 4
36	49	56	25 0	42 5	61	29 8

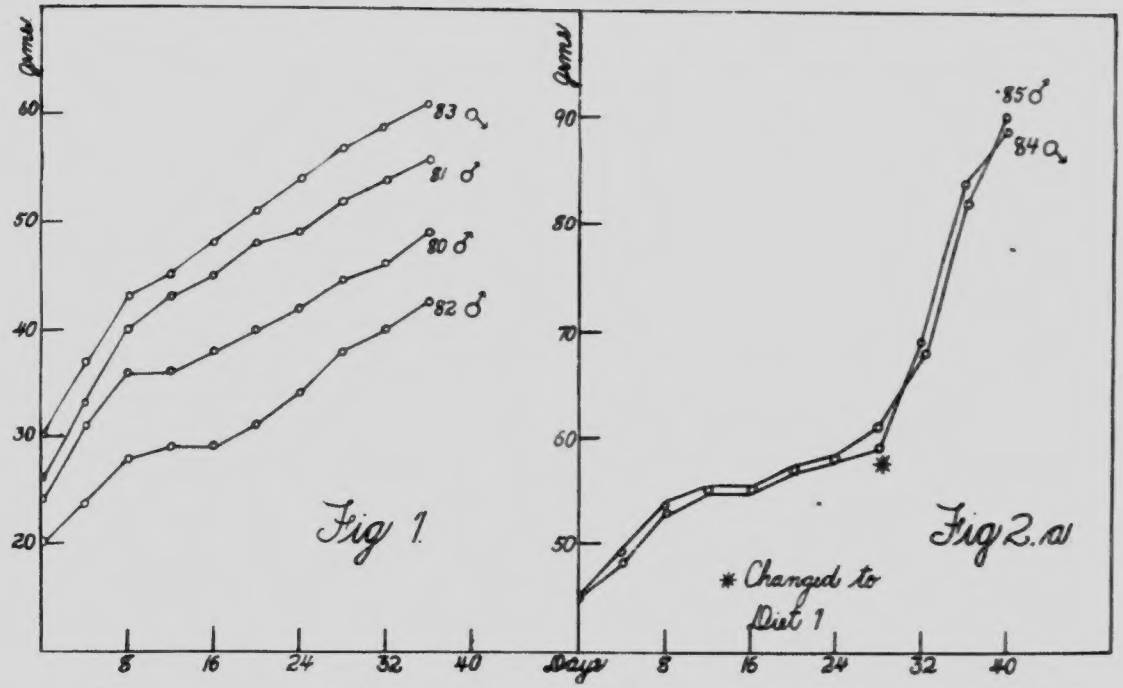


FIG. 1. Rats 80, 81, 82, and 83 were kept on a diet containing decomposed phosphotungstic acid precipitate from yeast.

FIG. 2 a. Rats 84 and 85 were kept first on a diet containing the residue from phosphotungstic filtrate of yeast. The marked deficiency was corrected when this addition was changed on the point marked on the chart to the corresponding precipitate.

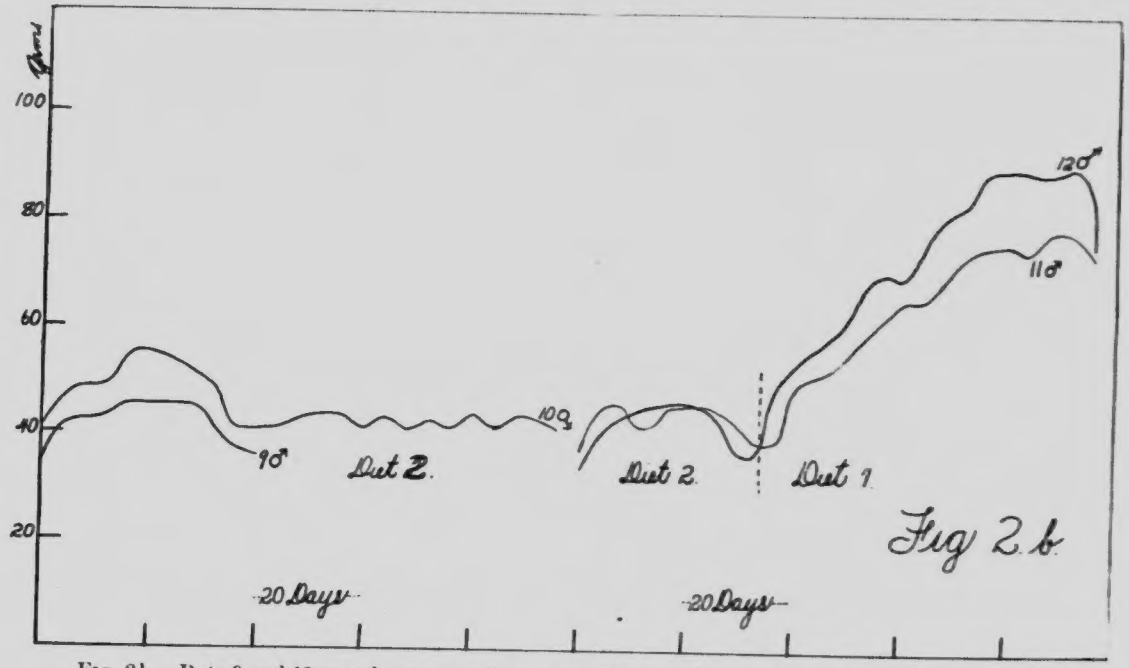


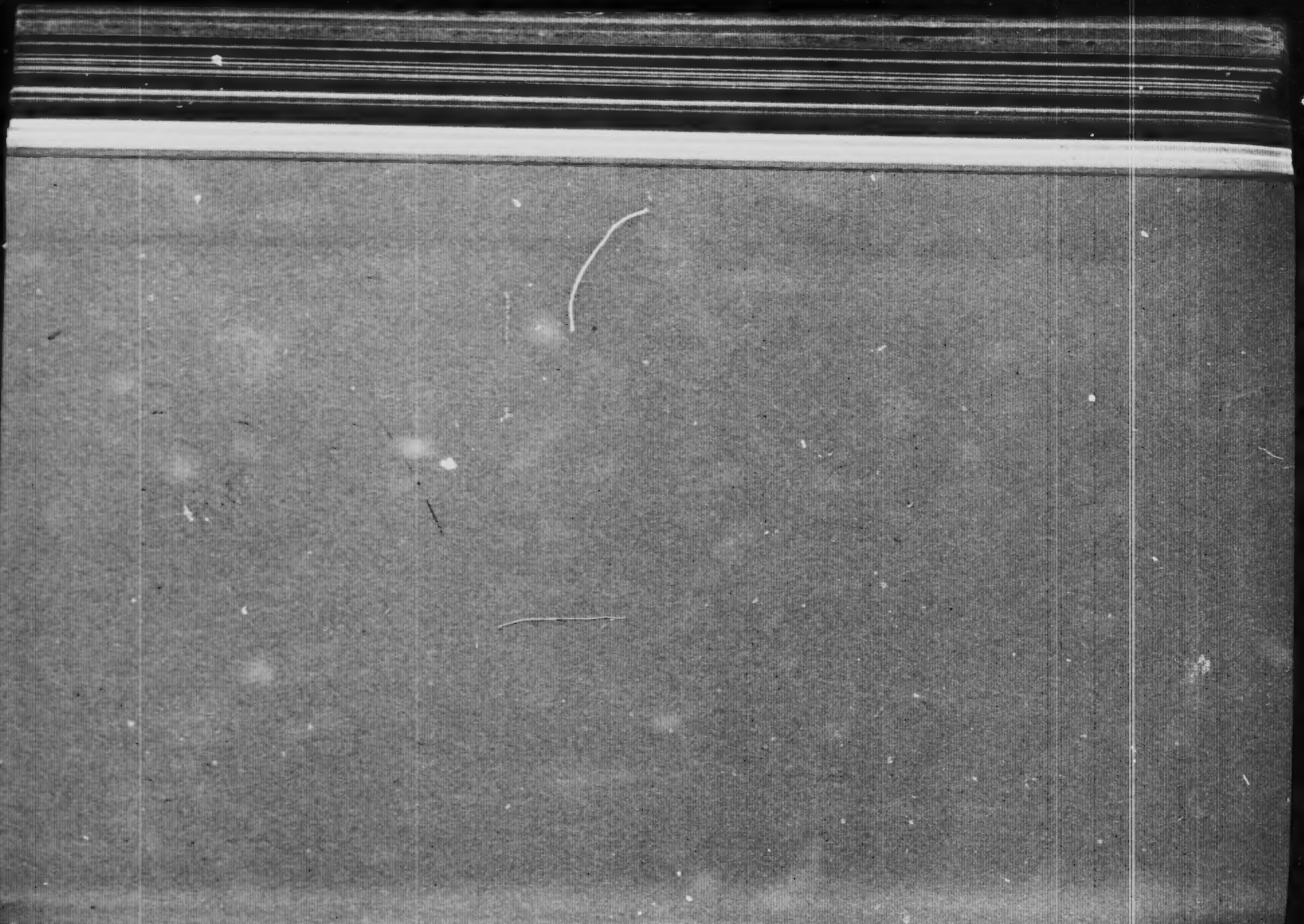
FIG. 2b. Rats 9 and 10 were kept on a diet containing phosphotungstic acid filtrate throughout the experiment. Rats 11 and 12 were changed to phosphotungstic precipitate at the point marked on the chart, with a marked improvement in growth and general appearance.

Rat 84. Female.
Rat 85. Male.
0–28 days Diet 2.
29–39 " " 1.

Days.	Weight.		Average daily food intake.
	84.	85.	
	gm.	*gm.*	*calories.*
0	45	45	
4	48	49	28.6
8	53	54.5	46.3
12	55	55	42.9
16	55	55	35.9
20	57	57	50.9
24	58	58	48.6
28	59	61	47.9
32	69	68	54.6
36	84	82	70.3
39	89	90	66.3

SUMMARY.

The fractionation of yeast with phosphotungstic acid shows that the growth-promoting substance is carried down with the precipitate and a large part of its activity is lost during the fractionation. The instability of this substance when fractionated with silver salts presents greater difficulty than that experienced during the fractionation of the beri-beri vitamine. It seems possible that both these problems can only be solved when more adequate methods are available.



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